Tropical Medicine and International Health

VOLUME 2 NO 12 PP 1170–1174 DECEMBER 1997

Early reduction of the challenge recovery rate following immunization with irradiated infective larvae in a filaria mouse system

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Summary The filaria *Litomosoides sigmodontis*, which develops a patent infection in BALB/c mice, was used to determine the fate of a challenge inoculum following immunization of mice with irradiation attenuated infective larvae (3 subcutaneous inoculations at weekly intervals with 25 L3 irradiated at 60 krad, and challenge with 25 L3 two weeks after the final immunization). The adult worm burden of vaccinated mice was reduced to 50% of that of controls although the pattern of larval migration and microfilaraemia were not affected. Necropsies showed that the increased killing of the filariae of the challenge inoculum occurred at the L3 stage within the first 2 days of challenge. This result draws attention on the protective mechanisms operating very early and probably in the subcutaneous region.

keywords *Litomosoides*, filaria, BALB/c mice, irradiated larvae, immunization

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Introduction

Immunization with irradiation-attenuated infective larvae has been shown to be a potent method for inducing partial protection against challenge infection in a variety of nematode species including filariae (Rao *et al.* 1977; Storey & Al-Mukhtar 1982; Yates & Higashi 1985; Lucius *et al.* 1986, 1991; Weil *et al.* 1992; Bancroft & Devaney 1993; Maréchal *et al.* 1994; Lange *et al.* 1994; Taylor *et al.* 1994; Schrempf-Eppstein *et al.* 1997). In contrast, vaccination with filarial homogenates or semipurified extracts (Carlow & Philipp 1987; Maréchal *et al.* 1994) or recombinant antigens (Lucius *et al.* 1994) have frequently been less successful in stimulating protection. Nevertheless some encouraging results have been recently obtained using filarial recombinant paramyosin (Li *et al.* 1993) and tropomyosin (Taylor *et al.* 1996).

In the present study, as a prelude to a detailed investigation of the mechanism of immunity evoked by irradiated L3 larvae of filarial nematodes, we investigated the early fate of challenge L3 larvae using the *Litomosoides sigmodontis*/mouse system. BALB/c mice infected with normal L3 larvae of this parasite develop patent infections (Petit *et al.* 1992; Maréchal *et al.* 1996) and the immune responses to such infections have been recently studied (Al-Qaoud *et al.* 1997; Maréchal *et al.* 1997). Partial protection against challenge inoculum can be induced by immunization with irradiated L3 larvae (Maréchal *et al.* 1994). This protection proved to be identical whatever the time lapse between the inoculation of infective attenuated larvae and the challenge (1–6 months; unpublished data in M.N.H.N. files number 11 LJ and 65 LI).

Materials and methods

Production and recovery of *L. sigmodontis* infective larvae from the vector *Ornithonyssus bacoti* were those previously described by Diagne *et al.* (1990) for *L. galizai*. Female mice were used (Petit *et al.* 1992) throughout the study. Infective L3 larvae were recovered from mites by dissection in RPMI 1640 and irradiated at 60 krad from a Cobalt 60 source (Institut Curie, Paris). One-month-old female mice ($n = 28$) were immunized with irradiated L3 by subcutaneous inoculation into the lumbar area alternatively in the left and right side at weekly intervals. Control mice ($n = 27$) were similarly inoculated with RPMI 1640 only.

Challenge (normal) infective larvae were recovered in RPMI 1640 supplemented with 20% calf serum, and inoculated (25 L3 per mouse) subcutaneously into the right side of the lumbar area two weeks after the last immunization.

Necropsies were performed 2, 10 and 60 days after challenge to determine the pattern of migration and fate of challenge larvae (Tables 1 and 2). Lymph nodes as well as the other internal organs were isolated and teased apart to favour the release of migrating larvae (technique in Bain *et al.* 1994). All dissections were performed in RPMI supplemented with 20% calf serum in which larvae survive for two days.

Table 1 Comparative evolution of filarial infection in vaccinated and control mice

Days between challenge/ necropsy	Number of mice	Dose (krad)	Number of larvae inoculated (L3*)	Microfilaraemia /10mm³ blood	% positive microfilaraemia	Number of filariae(F)	Mice with filariae (%)	F/L3† (%)	Protection (%)
2	8	60	3 × 25			**2.63 ± 1.4**	90	**10.5**	50
	8	0				5.25 ± 2.3	100	21	
10	10	60	3 × 25			**4.15 ± 3.6**	90	**16.6**	48.9
	8	0				8.13 ± 3.5	100	32.5	
60	10	60	3 × 25	1.33 ± 2.31	33.3	**3.30 ± 2.8**	100	13.2	57.8
	11	0		19.6 ± 20.23	80	7.8 ± 4.2	100	31.3	

*number of irradiated infective larvae inoculated; †percentage of challenge infective larvae developed into larvae or adults. The results are expressed as the mean ± SD. Values in bold represents statistical differences at 95% level between the vaccinated and control mice using the Mann–Whitney *U*-test.

Table 2 Distribution of larval and adult *L. sigmodontis* larvae in vaccinated and control mice

Days between challenge/ necropsy	Number of mice	Dose (krad)	Number of larvae inoculated (L3*)	Sub-cutaneous tissue	External lymph. system	Internal lymph. system	Peritoneal &vagino-peritoneal cavities	Heart	Lungs	Pleural & pericardial cavities
2	8	60	3 × 25	12.5	2.5	24.2	0	17.5	13.3	30 ± 36.1
	9	0		9.6	10.8	27.9	20.9	2.1	8.1	20.3 ± 23.4
10	8	60	3 × 25	0	0	0	75	0	0	25 ± 33.6
	6	0		0	0	0	52.2	0	0	47.8 ± 28.5
60	7	60	3 × 25	0	0	0	16.6	0	0	83.3 ± 40.8
	6	0		0	0	0	9.3	0	0	90.6 ± 16.8

*L3, number of irradiated infective larvae inoculated; External lymphatic system: inguinal, subiliac, axillary and neck lymph nodes: with associated lymphatic vessels; Internal lymphatic system: lumbar, iliac, mesenteric, sacral and thoracic lymph nodes with associated lymphatic vessels. The results are expressed as the mean of percentages of the total recovery ± SD.

Results

The mean worm burden of vaccinated mice at day 2 post challenge was 2.63 ± 0.5 compared with 5.25 ± 0.8 in the control group indicating 50% protection (Table 1). A similar level of protection was observed at days 10 and 60 post-challenge.

No differences were noted between the two groups of mice with respect to the pattern of migration of developing larvae (Table 2). Within 2 days of challenge, the majority of larvae had migrated away from the site of inoculation into the lymphatic and body cavities. However, at day 10 post-challenge, all larvae were recovered from the coelomic cavities.

No statistical differences between the two groups were found with respect to the mean microfilaraemia; frequency of patent infection; filarial cysts and coated filariae.

The morphology of worms recovered from the vaccinated mice did not differ significantly from those of the control mice, at any time. Two days after challenge, larvae had reached the third stage and were 1.0 mm long and 18 μm wide on average. Ten days after challenge, female and male larvae were at third moulting or fourth stages; 1.6 mm long and 30 μm wide. Sixty days after the challenge, female adults were 66.7 mm long and 139 μm wide and male adults were 21.7 mm long and 124.6 μm wide.

No abnormalities were observed in either female uterine embryogenesis (density of undivided eggs, divided eggs and uterine microfilariae) or male spicules development (Petit *et al.* 1992). The sex ratio was similar in both groups of mice.

Discussion

Various protocols with irradiated infective larvae have been used for vaccination against experimental filarial infections. They differ in the site of larvae inoculation, number of both larvae and inoculations, given doses of irradiation used for larvae attenuations, and intervals between inoculations. Only one study reported no protection, i.e. immunization with *Onchocerca lienalis* in CBA/Ca mice (Taylor *et al.* 1994). In most protocols, a reduction of the challenge recovery rate was obtained which varied from 30% to 100%. Similar protection is also obtained after repeated inoculations of non-irradiated normal larvae from various species (*B. pahangi*, Denham *et al.* 1983; *Monanema martini*, Wanji *et al.* 1990; *Acanthocheilonema viteae*, Eisenbeiss *et al.* 1994). Experiments with *A. viteae* suggest that protective immunity would be linked with a distinct larval stage, molting L3 (Eisenbeiss. *et al.* 1994) or L4 (Eisenbeiss *et al.* 1996).

Protection conferred by immunization with irradiated infective larvae is mainly analysed when filarial worms have reached the adult stage. However, in some studies, the effect of vaccination has been shown to affect early phase of parasite development (Hayashi *et al.* 1984; Bancroft & Devaney 1993; Taylor *et al.* 1994). In a longitudinal study using *O. volvulus* in BALB/c mice, protective immunity against the parasite was assessed during the first 28 days after the challenge (Lange *et al.* 1994). The challenge infection consisted of infective larvae contained in diffusion chambers implanted in the subcutaneous tissue. A protection of 64% was established between the third and the fifth day. It was associated with an increase of eosinophils in the chambers compared to control mice.

In the system using *L. sigmodontis,* where challenge infection is given by direct inoculation of the infective larvae in the subcutaneous tissue, protection was detected as soon as day two after challenge. This result is in agreement with the previous observations collected in the context of primary infections whatever the filaria-rodent model used (Bain *et al.* 1994; Maréchal *et al.* 1996). According to these authors, and for all models studied so far, the late recovery rate is determined during the two first days following inoculation. The surviving inoculated larvae are those which enter the lymphatic vessels, and thus directly escape destruction by the early inflammatory process initiated in the dermal-subcutaneous tissue (Bain *et al.* 1994; Maréchal *et al.* 1996).

In the vaccinated mice, the ability of the infective larvae to enter the lymphatic vessels is reduced. Thus the very early inflammatory process induced at the site of challenge deserves to be analysed in order to delineate the different effectors (among them eosinophils) and to characterize their antiparasite functions.

Acknowledgements

This investigation was supported by the Science and Technology for Development Programme of the European Community, Contract *n°* TS3-CT980037 and *n°* STD4-IC18-CT95–0026 and The Edna McConnell

Clark Foundation. We wish to thank the Institut Curie and especially Dr Averbeck for the utilization of the Cobalt 60 source. We are grateful to G. Milon, Institut Pasteur, Paris and I. Oswald, INRA-ENVT, Toulouse, for their valuable comments, and to N. Dogna who is in charge of the animal house.

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